

Population structure of *S. cerevisiae* from agave fermentations in Mexico

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Introduction

Mexico is a megadiverse country in terms of ecosystems, biological organisms, and cultures. In this context, **the production of traditional agave spirits blends the variety of local biological resources** (agave plants and microorganisms), with the **heterogeneity of traditional autochthonous practices**, to ferment and distill cooked agave hearts (Fig.1C). Most of the artisanal **mezcal production relies on open fermentations performed by yeasts and bacteria living between the factories and the variety of surrounding ecosystems**.

To study the evolutionary relationship, genetic diversity, and population structure of yeasts employed in production of agave spirits, we isolated and sequenced *S. cerevisiae* strains from fermenting agave juice of 62 different mezcal factories. Then, we compiled a dataset of 361 *S. cerevisiae* genomes:

- 154 from our agave collection
- 23 from tequila reported in Lachance, 1995 and Padilla *et al.*, 1994
- 168 reported in Peter *et al.*, 2018, (whole Mexico and French Guiana)
- 16 from Brazil reported in Barbosa *et al.*, 2016

Results

Most mezcal agave strains group in a new cluster

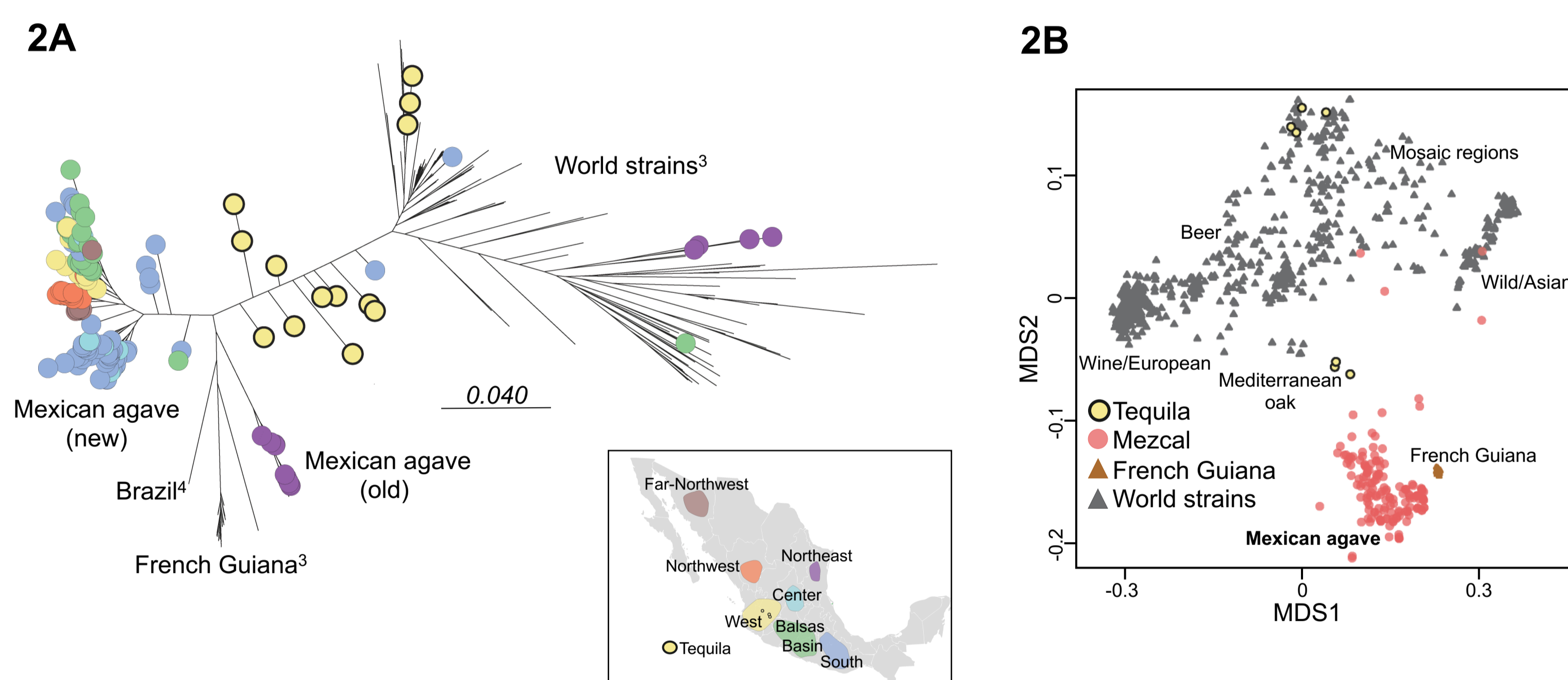


Fig. 2) A. Maximum likelihood tree using the program RAXml with the subset of 361 genomes using 961,937 biallelic SNPs. The tip branches with agave strains are colored based on the isolation region depicted in the inset. **2B. Multidimensional scaling analysis** using the program plink v1.9. Graph of the 1st and 2nd components, using 29,690 biallelic SNPs from 1,017 *S. cerevisiae* genomes, showing that the agave strains form a separate group.

Agave spirits production

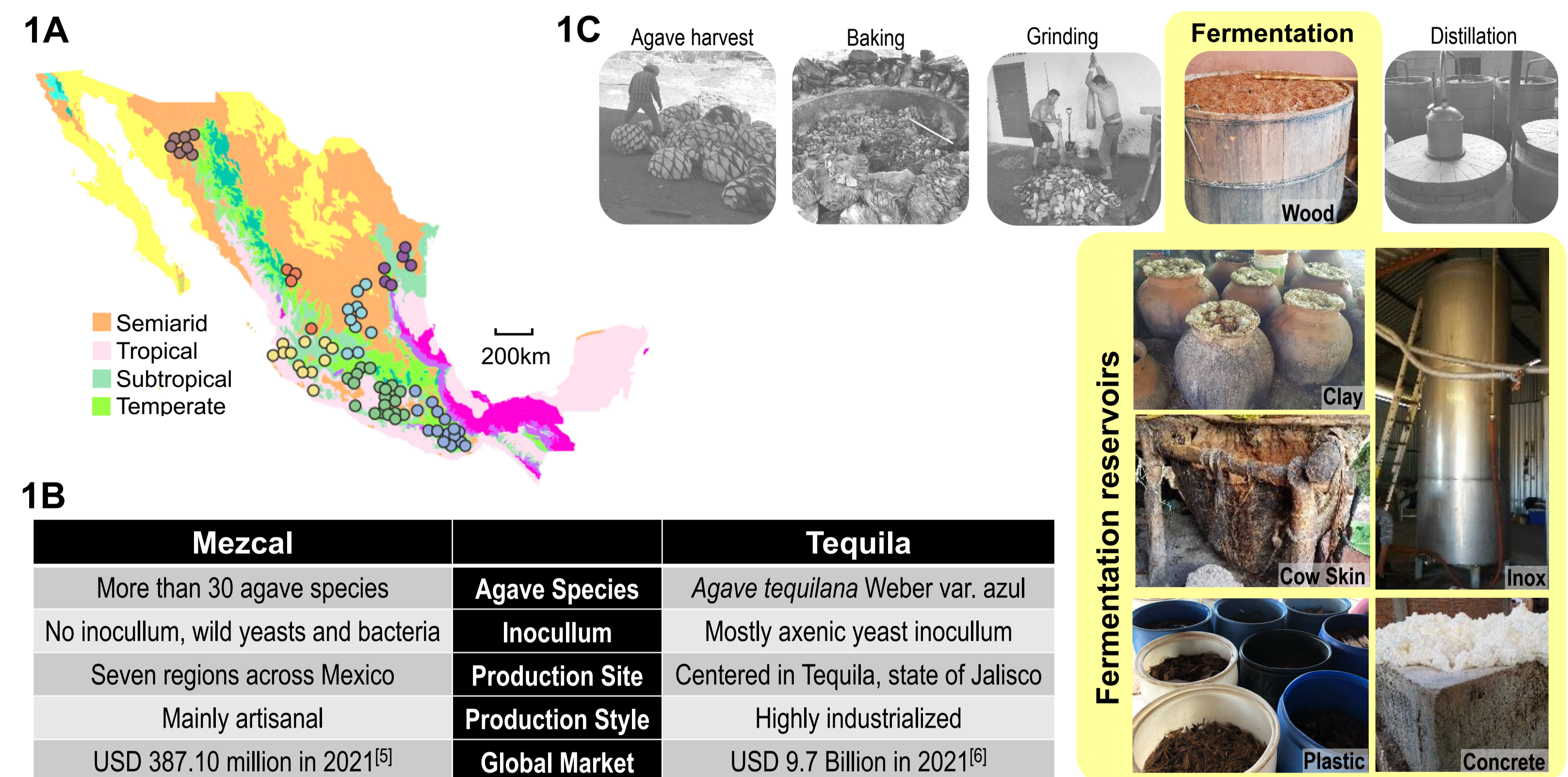


Fig. 1) A. Mexican traditional agave spirits are produced in diverse contexts. This map depicts the locations in which agave spirits are produced, in the context of different ecosystems. **1B. Differences between mezcal and tequila.** **1C. Artisanal mezcal agave fermentations occur in open reservoirs without inoculum.** This figure depicts the main steps of agave spirits production and various reservoirs with ferment.

The Mexican Agave *S. cerevisiae* population is highly structured

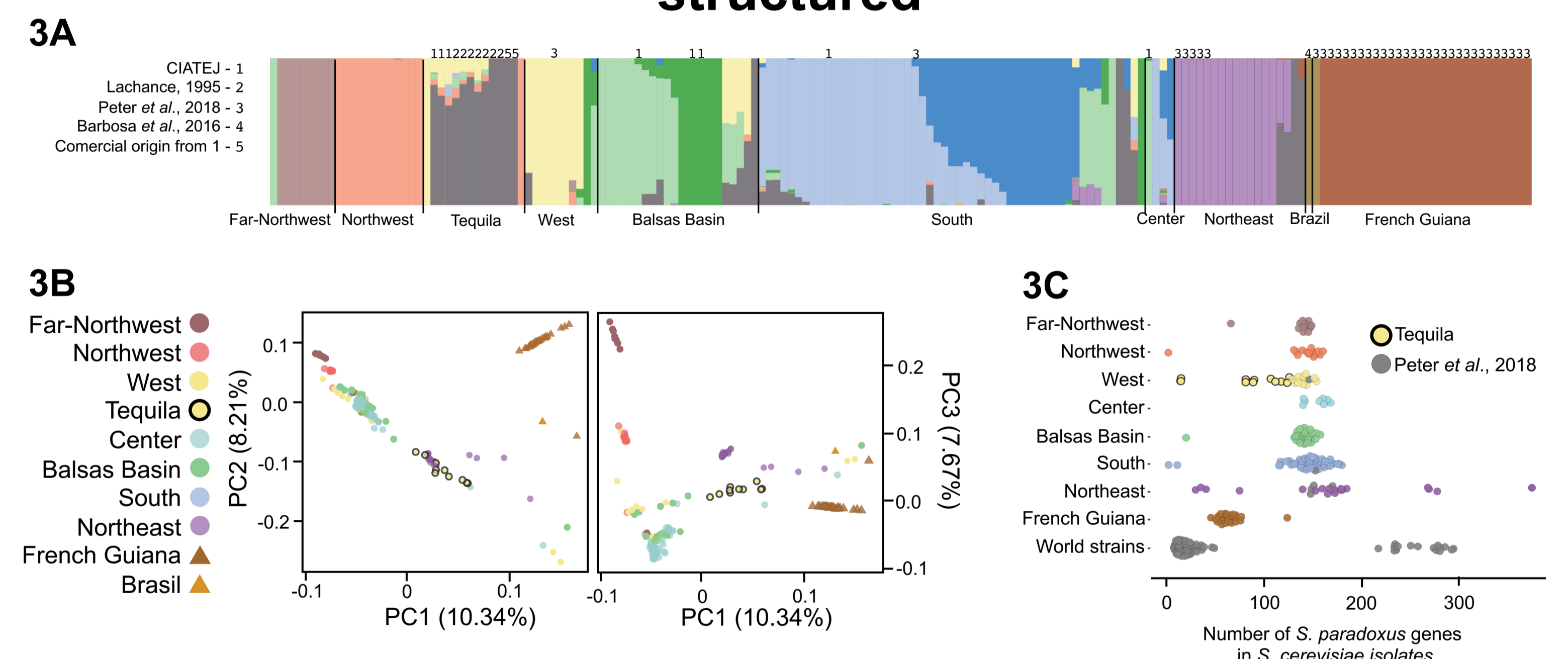


Fig 3) A. Inference of population structure using the programs Admixture and pong on 173 *S. cerevisiae* Mexican agave, Brazil and French Guiana isolates with a set of 36,794 markers filtered by LD with 500 bp and $r=0.35$, assuming $K = 11$ subpopulations, with 15 random seeds. Each rectangle on the x axis represents one strain, and its colors represent the fraction of the genetic material in each strain assigned to each of the 11 clusters. **3B. Principal component analysis** of the same strains and markers as panel 3A. **3C. Number of genes from *S. paradoxus*** for each strain, separated by regions.

Highlights

- The genomes of most mezcal *S. cerevisiae* strains group in a new cluster.
- Some migrant strains isolated from Tequila, group with bread strains, and interestingly some others show signatures of admixture between bread and West mezcal strains.
- Population structure analyses show that genomic variation correlates with the geographical regions with some admixture between subpopulations.
- Introgressions of *S. paradoxus* in these Mexican Agave strains are prevalent. We even found one mezcal strain with more than 370 *S. paradoxus* genes.

Agave fermenting yeasts in mezcal are genetically different from those of tequila

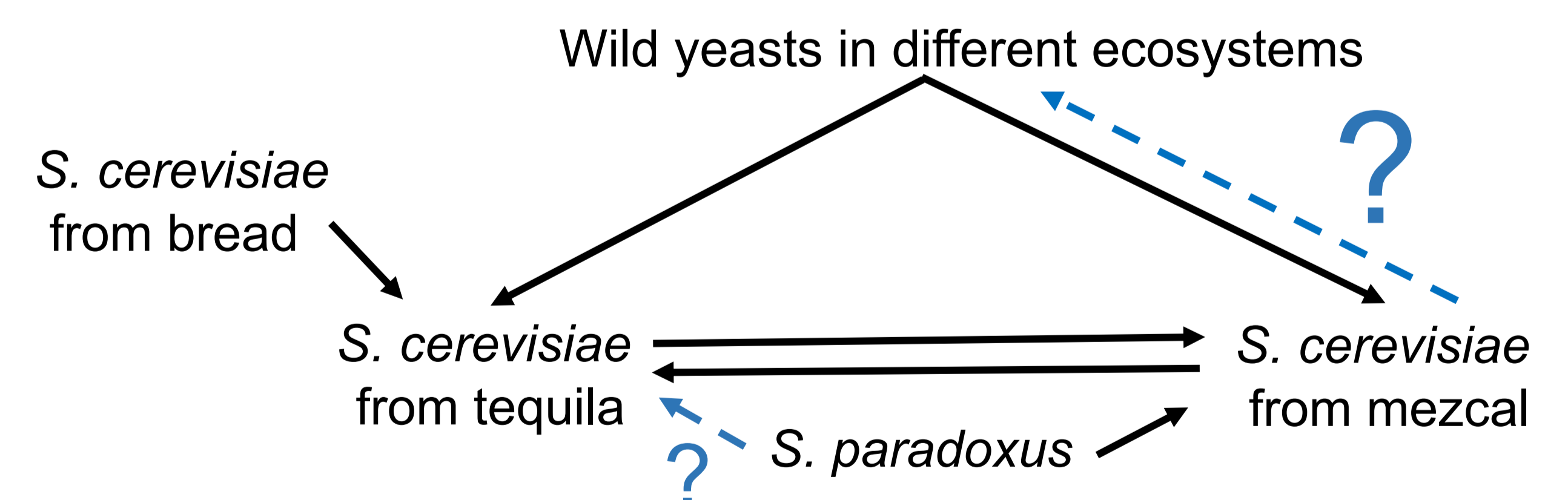


Fig 4) Sketch of possible model summarizing our observations and future research

Perspectives

- Estimates of genetic diversity per group are work in progress
- We also plan to evaluate the impact of introgressions in our findings
- Increase the sample size of tequila isolates

Acknowledgments

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References

- [1]. Padilla *et al.* 1994. Catálogo de la Colección de Cultivos Microbianos. CIATEJ, Jalisco, México
- [2]. Lachance. 1995. Yeast communities in a natural tequila fermentation. *Antonie van Leeuwenhoek* 68, 151–160
- [3]. Peter, *et al.* 2018. Genome Evolution across 1,011 *Saccharomyces cerevisiae* Isolates. *Nature* 556 (7701): 339–44
- [4]. Barbosa, *et al.* 2016. Evidence of Natural Hybridization in Brazilian Wild Lineages of *Saccharomyces cerevisiae*. *Genome Biology and Evolution* 8 (2): 317–29
- [5]. Global mezcal market – industry trends and forecast to 2029. *Mezcal Market Size, Share, Report, Impact, Price, & Forecast By 2029*. (n.d.). Retrieved September 2, 2022, from: <https://www.databridgemarketresearch.com/reports/global-mezcal-market>
- [6]. Vantage Market Research. (2022, August 24). Global Tequila Market. Retrieved September 2, 2022, from: <https://www.globenewswire.com/en/news-release/2022/08/24/2503914/0/en/Global-Tequila-Market-Size-Worth-USD-13-5-Billion-by-2028-at-5-70-CAGR-Vantage-Market-Research.html>

